



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

VIRGINIA LAW REVIEW

VOL. II.

APRIL, 1915

No. 7

BLOOD SPOTS AS EVIDENCE IN CRIMINAL TRIALS.

TO BE known as a person who will "run with the hare and hunt with the hounds" is desired by no one who esteems his reputation for sincerity and stability of character, and yet this expression may be applied aptly, though without disparagement, to some officers of the law who employ modern methods for the detection of crime. The value of the hound for obtaining evidence is now equaled for some purposes, or even exceeded, by the value of the hare, or rabbit, and the testimony furnished by the latter is no less conclusive and is even more delicate than that given by the hound. The hound may reveal the intricate wanderings of a murderer from the scene of his crime; the hare may be used as a medium through which the bloody stains of the victim may be made to cry aloud.

In the following pages an account will be given of a method which utilizes hares, rabbits or other suitable animals in order to obtain valuable evidence from blood spots or from other stains of similar nature.

If articles such as knives or clothing, introduced as evidence during a trial, have suspicious stains on them, several well-known tests may be employed to ascertain whether or not the stains are due to blood. These tests, however, give only a limited indication of the kind of animal from which the blood came, and it may be highly important to determine whether human blood or blood of a lower animal produced the stain. There are two or three procedures of rather recent development in medical science which are valuable for this purpose. Of these the one best

known and most thoroughly tried out is called the "Forensic Test for Blood" or the "Precipitin Test."

THE "PRECIPITIN" PHENOMENON.

If blood from any animal is allowed to flow into a glass and is put aside to stand, a clot forms which is of jelly-like texture, dark red in color, and contains practically all of the red corpuscles, or coloring matter in the blood. The clot gradually contracts to smaller size and separates itself from the clear, pale yellow, fluid part of the blood which is called "serum" or "blood serum." If serum obtained with proper precautions from a human being is inoculated by means of an inoculating syringe into the tissues of a healthy rabbit, the rabbit will appear to be unaltered by the treatment. Even after several doses of human serum have been inoculated into a rabbit during a period of two or three weeks, the animal will appear absolutely unaltered as it moves about in its cage, but it can be shown that certain delicate changes have occurred in its system as the result of the inoculations. We may draw some blood from the inoculated rabbit, separate the serum from the corpuscles and perform a very striking test with the serum. If two or three drops of this rabbit's serum are placed in a small glass tube with a little water containing a trace of table salt (salt solution), the mixture is perfectly clear and will be slightly yellowish or perfectly colorless according to the proportion of rabbit's serum. If, now, a drop of diluted human serum is added to the fluid in the tube a change occurs. The fluid no longer remains clear but little by little becomes cloudy and turbid, and soon small whitish flakes or grains settle from the fluid and form a sediment at the bottom of the glass tube, the fluid again becoming clear and remaining so. In other words, by bringing together in proper dilution a drop of human serum and a drop of serum from a rabbit, which had previously received inoculations of human serum, a clouding occurs in the mixture with the formation of a precipitate. This phenomenon is spoken of as the "precipitin reaction." The rabbit's serum is said to contain "precipitins" which, interacting with some ingredient of the human serum, cause the formation of a "precipitate." The rabbit's serum is sometimes

spoken of as "precipitin serum," or as "antihuman" serum. The rabbit which has received the inoculations of human serum may be spoken of as a "humanized rabbit," or as an "antihuman rabbit."

It must not be supposed that rabbits are the only animals which will produce precipitins after inoculations. On the contrary, practically all healthy animals have this power. Rabbits are usually employed for the production of the precipitating serum because they are such convenient and inexpensive animals, and because it will seldom happen that the blood stain under suspicion could itself have come from a rabbit. Sheep, goats or chickens are occasionally selected to receive the inoculations, with results as satisfactory as when rabbits are employed.

How striking the precipitin reaction is does not appear until the test is repeated with serums from other rabbits when it will be found that ordinary rabbits give no precipitate when their serum is mixed with human serum, while the reaction is invariably produced when human serum and humanized rabbit's serum are properly mixed. In other words, it can be shown that the rabbits which receive human inoculations actually change in regard to the properties of their serum, and possess properties not possessed by untreated rabbits.

SPECIFICITY OF PRECIPITINS.

A further test can be performed. If several rabbits are treated with inoculations of human serum, while a second group of rabbits are treated similarly with inoculations of bovine serum, while several others are treated with inoculations of chicken serum, tests can be performed after a proper interval with the serum from each of these animals. It will be found that the mixture of human serum with the serum from rabbits treated with bovine or chicken serum will remain clear, while in every case the mixture of human serum with serum from humanized rabbits will become cloudy and give a precipitate. In the same way, if bovine serum is added in another series of glass tubes to serum from the treated rabbits, it will be found that the bovine serum regularly causes a clouding and precipitate in the tubes containing serum from bovinized rabbits but in no others. Sim-

ilarly chicken serum will give the precipitin reaction only with serum from the rabbits previously treated with chicken serum.

Not only will the humanized rabbit serum give a precipitate with the serum of that individual who furnished the blood for the original inoculations of the rabbit, but also it will give a precipitate when mixed with serum from any human creature. We may say, then, that the property of forming a precipitate in the presence of humanized rabbit serum is distinctive of human serum, as contrasted with serums from other animal species. In other words, the precipitin reaction with humanized rabbit serum is "*specific*" for human beings. Likewise the reaction with bovinized rabbit serum is "specific" for cattle; the reaction with galinized rabbit serum is "specific" for chickens.

The "specific" value of the precipitin test is now well established, and this method is used to supplement zoölogic methods in determining species relationships. In one series of experiments Nuttall proved the specific value of the precipitin test with 586 different species of blood. It is remarkable that the crystalline lens from the eye does not behave in the same "specific" manner as other tissues do. Extracts of human lens will not precipitate with humanized serum. A rabbit inoculated with extracts of lens will give a serum which precipitates lens-extract from any animal but gives no precipitate with other organ extracts from human beings.

A properly prepared rabbit serum will cause a precipitate when mixed with human serum, even in great dilution, but it is also almost equally active in precipitating extracts from the more solid tissues of the body. A blood clot or a piece of human flesh may be extracted in water, for example, and the resulting fluid will be precipitated by humanized rabbit serum. In this way it is possible to recognize the human origin of any tissues, secretions, or discharges which contain traces of human albumin. Even albuminous human urine can be made to yield a precipitate with humanized rabbit serum.

To prepare a precipitating serum, it is necessary to inoculate fairly large amounts of material. One or two ounces of human serum distributed over three or four inoculations during three weeks will usually suffice to produce a strong precipitating serum.

In the same way, if extracts of solid tissues are inoculated into the rabbit, equally large amounts will be needed. A precipitating serum will result from inoculations of suitable quantities of almost any material from the body provided only that the inoculated matter be albuminous or proteid in character. Materials which are not albuminous are usually not suitable for use in the production of precipitins.

Not all precipitating serums are of equal value, in fact it requires care in the selection and treatment of the animal inoculated in order to procure serum of strength sufficient to give precipitates with very small quantities of the kind of material inoculated. With care, however, humanized serums can be prepared which will give a precipitate with infinitesimal amounts of human material. It is not uncommon to obtain a humanized rabbit serum which will reveal the presence of human blood diluted 1,000 or 5,000 times, and in rarer cases human blood may be detected when diluted 10,000 times. In one instance cited in the literature of this subject, 1/20,000th gramme of material gave conclusive specific precipitin tests. In another instance, 1/200,000th gramme was sufficient.

It may happen that the inoculated animal will not yield a sensitive precipitating serum even after receiving adequate doses. There is a great difference between one rabbit and another in this respect which can not be discovered except by experiment. Sometimes the serum drawn from a rabbit will be a little cloudy, or milky. Often this may be avoided by drawing the blood from the rabbit after the animal has had no food for half a day or a day. If the serum is to be utilized for precipitin tests, it must be very sensitive and perfectly clear. Filtration will sometimes clear a cloudy serum.

A humanized serum, once prepared, will keep for a long time—several months. It is best to keep it in sealed glass tubes in the refrigerator, though this is not absolutely necessary. A humanized serum will often act well even after moulds or bacteria grow in it. In every case, whether the humanized serum be fresh or old, it must be carefully tested with dilutions of normal blood from human beings and from lower animals, and the operator must be perfectly certain, from such tests, that the

humanized serum is thoroughly satisfactory, before he makes the crucial tests with the blood spots given him for examination.

SIGNIFICANCE OF PRECIPITIN REACTION.

The precipitin reaction is certainly a remarkable phenomenon. Why should the serum of one animal, A, acquire the property of precipitating tissue extracts, or serum from any other species of animal, B, as a result of the previous insertion into the body, A, of organic matter obtained from any member of the species B? It would not seem likely, at first thought, that any process resembling the inoculation of tissue from one animal into another, could occur in nature. But if this reaction is not of frequent use to animals, why should we find that the ability to form precipitins is so universally present throughout the animal kingdom? Is it possible that such a widely present property is only a meaningless freak of nature?

We get at least a partial answer to such queries from a study of bacterial infections. If a person comes down with typhoid fever, a period of illness follows and usually the patient recovers. After recovery it is found that the patient is less apt to have typhoid fever than other people in the community who have never suffered from the disease. Similarly with scarlet fever and several other infectious diseases, one attack protects the patient from subsequent attacks for a longer or shorter time. We speak of these protected individuals as being "immune" or "immunized." We know that this protection may be secured in some instances without the occurrence of actual attacks of the disease. For example, in the case of typhoid, if a healthy individual receives inoculations of killed typhoid germs he can be protected or "immunized" so that he is practically free from danger of typhoid fever for years thereafter.

If, now, we try to determine upon what this protection depends, we find that the tissues and body fluids of the immunized person have acquired certain properties not possessed by the tissues and fluids of a person who has not been so immunized. By virtue of these properties the tissues and fluids are able to destroy the typhoid bacteria much more quickly than would

otherwise be possible and to neutralize to a certain extent some of the poisons of the bacteria. We need not concern ourselves with all of the new properties acquired by the individual through immunization. Suffice it, that, among other powers, the serum of the individual acquires the power to form a precipitate when mixed with typhoid organic matter. If a few drops of serum from an immunized person are put in a glass tube with some fluid in which typhoid bacteria have been growing, but in which the typhoid germs themselves need not be present, there gradually develops a cloudiness of the fluid and the formation of a sediment or precipitate exactly as occurred in the cases previously mentioned. It can be shown that this reaction is specific in the same sense as we have seen that the reaction with humanized rabbit serum is specific; the serum from the typhoid-immune subject giving a precipitate with material from any typhoid germs, but failing to give a reaction with bacteria unlike typhoid bacilli. What has been said of typhoid bacteria and their precipitins might be repeated of other infectious bacteria; in recovery from an infectious disease, or in immunization, the patient regularly develops the property of forming a precipitate with his serum when those bacterial substances which have been brought into his body are mixed in a glass tube with his serum. We may suppose, then, that the development of precipitating power is a part of the protective mechanism by which the body overcomes infections, and this may lead us to understand why the precipitin-forming power is so common among animals. We do not yet, however, understand clearly how the immunized animal is protected by the possession of precipitating powers.

This peculiar reaction is not limited in its application to human and animal albumins, and to bacterial proteids, but may be utilized for the detection of any proteid either of animal or plant. In every case the kind of proteid to be examined is first injected into animals in rather large amounts, and after three or four weeks the serum of the animal will precipitate even minute traces of the inoculated kind of proteid. In this way serums may be obtained which will react with egg albumen, milk, proteids of grain, etc., etc. A serum precipitating cow's milk will also pre-

precipitate extracts of cow's tissue, but will not precipitate milk from other animals.

"BLOOD RELATIONSHIPS:" "MAMMALIAN REACTION."

It can be seen from what has been stated that the precipitin reaction will not allow one to decide which of several body albumins is being tested, nor will it serve to distinguish between different individuals of the same race. Some workers claim that they can accomplish the latter object, but it is not proven beyond doubt. Occasionally the combined use of the microscope and the precipitin test will settle the former point. In general, however, after inoculations into a rabbit of one kind of albuminous material from the body of an animal, the rabbit's serum will precipitate all kinds of albumin from that animal species. Thus, for example, after inoculations of extracts of bovine muscle tissue into a rabbit, the rabbit's serum will precipitate solutions of bovine muscle, or liver, or heart, or kidney, or blood, etc.

After a rabbit has received inoculations of human serum it is customary to draw some of its blood, obtain the clear serum, and determine its precipitating power. This test is performed by taking several glass tubes, placing in each a constant quantity, let us say two drops, of the rabbit's serum, and adding graded amounts of human serum. In the first tube is put a dose of ten or twenty drops of human serum diluted 100 times (that is, a 1 : 100 dilution of human serum). In the second tube a dose of 1 : 200 dilution is placed; in the third a dilution of 1 : 500, and so on, the tubes at the end of the series receiving extremely small traces of human serum, to mix with the two drops of rabbit's serum. Within twenty minutes the tubes are examined. A precipitate will be present in the first few tubes containing the larger quantities of human serum, but will be absent from the ones with extremely little human serum. If the precipitate appears in tubes containing human serum in a dilution of 1 : 1,000 but does not appear where the dilution is greater, the humanized rabbit serum will be designated as a "1 : 1,000 antihuman serum."

If such an antihuman serum is tested by mixing two drops in glass tubes with extracts from tissues of goats, cows, sheep, dogs, etc., usually none of these extracts will give a precipitate with the antihuman serum. Some antihuman serums, however, especially very strong ones, will give precipitates with the extracts of the other animals. This reaction, called the "mammalian reaction," is never confusing as it never occurs when the tissue extracts are much diluted. In other words, the reaction is not a delicate one.

A much more definite precipitation occurs if antihuman serum is mixed with dilutions of monkey's blood. In this case the precipitate develops even with fairly high dilutions of the monkey's blood. However, monkey's blood will never give a precipitate with antihuman serum in anything like as great a dilution as human serum will. This phenomenon is supposed to show a "blood relationship" or "proteid relationship" between human beings and monkeys, and confirms other zoölogical evidence. The same kind of "proteid relationship" is found to exist between sheep and goats; between typhoid bacteria and nearly related bacterial forms and in many other instances. Reactions of this nature might seem to weaken the value of the precipitin test, but in fact they do not do so at all if the test is performed carefully. If an antihuman serum is tested with human blood, using smaller and smaller quantities of human blood in successive tests, the test will be positive—that is, a precipitate will form—even when very small amounts of human blood are used—possibly in a dilution of 1 part human blood to 3,000 parts of salt solution. In a test of the same kind, monkey's blood diluted 1 part to 1,000 parts salt solution may precipitate the humanized serum, but smaller quantities of monkey's blood will fail to act. By employing a sufficiently strong humanized serum, and testing it against sufficiently great dilutions of the blood for examination, it is possible not only to prevent confusion from "proteid relationships" but even to confirm the results of tests with human blood. When the quantities are carefully measured, the precipitin test may reveal slight differences between the serums of the several branches of the human family—Caucasian, Chinese, Malay, Negro, etc.—

but it is doubtful whether these differences are clear-cut enough for use in trials.

PRACTICAL APPLICATION OF PRECIPITIN TEST.

We may best get an idea of the rôle which the precipitin test can play in the course of a trial, by illustrating with an imaginary case. Let us imagine that a murder has been committed. Suspicion attaches to a man, and on examination dark spots are found on his clothing. After noting the location and extent of the spots, the clothing should be locked up in a safe for delivery to the medical expert. Usually a doctor or a bacteriologist familiar with the methods employed in studies relating to immunity will be able to make the forensic precipitin test. The medical examiner—if the spots are large enough for all tests—should first decide by the standard tests whether the stains are due to blood, and, if a recently described method proves reliable, he may be able to estimate with fair accuracy the age of the spots. If the spots are found to be blood, the garment should be locked up in a safe until needed. The medical expert, knowing as much as possible of the habits of the accused, will inoculate a series of healthy rabbits or of other suitable animals. Into some of these rabbits he will inoculate human serum, into others the serum of such domestic animals as the accused may have had opportunity to come in contact with. After an interval of usually three or four weeks the inoculated animals are ready for use. Samples of the serum of each one will be tested against the same kind of material the animal was inoculated with and the precipitating strength of each serum will be determined. Only such animals as yield a very sensitive precipitin-serum will be utilized. From these animals the serum will be drawn and carefully bottled for use and preserved under proper conditions. Each serum will then be tested against blood from each of the species which might conceivably have furnished the blood on the clothing. The result of each test is noted. Finally when all these preliminaries, which occupy some time, have been finished, the medical expert removes the clothing from the safe, cuts out a stained part, dissolves the stain in

water, makes the extract perfectly clear by filtering it, dilutes the extract with salt solution to a proportion estimated at 1 part of stain to 1,000 of fluid and mixes in glass tubes some of the resulting dilution with each of the specific serums prepared by inoculating the rabbits. The results in all the tubes are observed and compared. A precipitate will form in only one tube, namely, that tube which contains precipitins specific for the kind of organic material represented by the blood clot; if the blood stain contains human blood, a precipitate will form only in the tube containing the humanized rabbit serum. If no precipitate forms in this tube, the blood on the clothing is, in all probability, not human blood. Records of all the tests should be made for use at the trial. Simultaneously tests should be made with extracts of parts of the clothing free from stain. These should give no precipitate.

USES AND LIMITATIONS.

This test has been standardized and made a part of the regular court procedure for suitable cases in Germany and Austria, and it has been used occasionally in the courts of England, Italy, Spain, Norway, Roumania, Egypt and the United States. It has been tried out thoroughly during the last twelve years, and has proved a highly satisfactory test. It is successful with blood stains even many years old, though not with extracts of ancient mummies; with putrefying blood; with blood on nearly any fabric used for clothing or for house furnishing; with stains on wall paper, and at times with blood mixed with earth. The reaction cannot be obtained with blood spots washed out in water, nor where the material containing the blood is strongly acid or alkaline. Some metallic salts and some other chemicals also may block the reaction. If the blood stain has been heated, the ordinary precipitin test is not applicable, but a modification recently described may be used to obtain precipitins even after the blood has been heated to the boiling point. Apparently blood stains in the tropics lose their power to precipitate more rapidly than do similar stains in temperate zones.

The cases mentioned in the medical literature show that the precipitin reaction has served to establish the guilt of suspects

in some cases, and in others, where the test gave a negative result, has helped to clear the prisoner from suspicion.

Unquestionably the chief forensic use of the precipitin reaction hitherto has been in murder trials. In a few cases, however, the same method has been employed in the detection of food adulterations. By this method it can be determined what kinds of meat are sold in market; what kinds are in sausages; etc., etc.

The precipitin test described above is the best known of the so-called "immunity tests" or "serological tests" employed for the determination of the nature of albuminous materials, but there are also other tests which have even a greater sensitiveness, and which may be employed for the same purposes. These tests, called the "complement fixation" test and the "anaphylaxis phenomenon," are very sensitive and may have a limited field for use in the law courts, but hitherto neither of them has been utilized to any great extent for forensic purposes.

Harry T. Marshall, M. D.

UNIVERSITY OF VIRGINIA.